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TITLE: Phase I Trial of Adenovirus-Mediated IL-12 Gene Transduction in Patients with Recurrent Locally Advanced Prostate Cancer Following Therapy

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13. SUPPLEMENTARY NOTES

14. ABSTRACT: Background: Patients with radiorecurrent prostate cancer have few options. Gene therapy may define a treatment option of both local and systemic value. Pre-clinical studies using adenovirus-mediated (Ad.) transduction of IL-12 (Ad.mIL-12) in a metastatic model of prostate cancer resulted in local growth suppression, survival enhancement and inhibition of pre-established metastases. The basis for these activities include the induction of both innate (neutrophils & NKs) and acquired immunity (T cells). Objectives/Hypohesis: On the basis of these results, we propose to explore the use of Ad.hIL-12 in patients with clinically localized radiorecurrent prostate cancer in a Phase I trial to explore the safety, induction of immune responses and efficacy following therapy. Specific Aims/Study Design: In Aim I patients will be placed in escalating dose cohorts with the primary endpoint of the maximum tolerated dose as determined by physical examination, laboratory values of bodily functions and evidence of IL-12 gene transduction by measurement of serum by ELISA.. In Aim 2 addditional safety data will be recorded through measurement of serum levels of the pro-inflammatory cytokines, TNF-α, IFN-γ and IL-16 by ELISA. In Aim 3 peripheral blood mononuclear cells (PBMCs) will be screened for the induction of T cells, which target the prostate antigens, PSA and PAP via an ELISPOT assay. In Aim 4 evidence ofefficacywillbesuggestedfrommonitoringofserumPSA.

15. SUBJECT TERMS
Cancer Therapy: gene therapy, adenovirus, tissue specific gene expression, Fas/FasL gene expression

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Since the annual report of last year (2/2005), the only regulatory hurdle left was a Mount Sinai requirement to have an independent process of regulatory oversight over and above that of the DSMB and medical monitor. This is an institutional requirement for all gene therapy protocols performed at this facility – reports from this audit, performed after each dose cohort would be forwarded to the IRB, PI of the trial and the DSMB. The original intent of the Medical center was to have trained auditors available for investigators to hire for these protocols. However, with several monetary changes within the School, this service was no longer available and we thus were left to find such a service on our one. We identified a funding source through the Deane Prostate Health & Research Center to cover this expense, which was not approved in the original budget and after interviewing 2 vendors, we were able to negotiate a suitable contract with one which was approved by our GCO.

We have been actively screening patients for over a year. To date we have screened 11 suitable patients, ie those with radiorecurrent disease as defined by a rising PSA following definitive radiation therapy who are presently not on hormonal therapy. An important part of the screening process is a needle biopsy of the prostate to confirm the presence of disease in the prostate. Two patients refused to have a biopsy and went on to other treatments. The remaining 7 were biopsied but only one was found to have cancer. This patient decided not to enroll in this trial. Based on the literature this was a surprising finding to us, that patients experiencing apparent radiorecurrent disesase had negative prostate biopsy, indicating that while local disease appeared to be control, failure was a as yet clinically undetectable systemic failure. This may reflect the trend in improving the quality of radiation therapy in this country through the use of 3D-conformal and IMRT to boost the dose of radiation to the prostate to over 72 Gy and/or better quality seed implants and/or the use of combination therapies of either seed plus external beam which results in better local control but distant failure.

To this end the protocol has been amended (see below) to also include patients who had their locally advanced prostate cancer treated with hormonal ablative therapy initially and are now experiencing treatment failure as documented by a rising PSA. Patients may have lymph node positive disease but no bone disease. We are about to complete accruing patients to a competing Phase III trial for this group which has done well and thus expect that this change in protocol allow us to accrue patients and complete this Phase I trial.

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I. Rationale & Objectives

Pre-clinical work has demonstrated local and systemic growth effects of a single injection of Ad.mIL-12 in a pre-established, metastatic model of prostate cancer. With the lack of effective therapies for men who have failed definitive radiotherapy or who have locally advanced cancer despite hormone ablative therapy, a Phase I clinical trial of Ad.mIL-12 will seek to take advantage of both local and systemic growth effects. Vector injection will be performed by a transrectal ultrasound (TRUS) guided needle into the prostate. Endpoints will be in order of significance, toxicity, assessment of an immune response and appraisal of tumor response.

1**:** To study in a Phase I clinical trial the safety of intraprostatic injection of a replication incompetent adenovirus expressing hIL-12 in patients with radiorecurrent or hormone recurrent locally advanced prostate cancer prostate cancer.

2**:** To assess serum levels of pro-inflammatory cytokines before and after vector injection.

3 To assess T cell responses pre and post-IL-12 treatment against prostate antigens.

4: To assess changes in PSA levels as a surrogate marker for prostate cancer following Ad.hIL-12 gene therapy.

II. Selection of Patients - Patient Eligibility

If the highest dose cohort proposed is achieved, the study will accrue a total of 24 patients. The population targeted is men who underwent radiation therapy, either external or seed implant, as definitive therapy for prostate cancer who have now suffered treatment failure. Patients for this trial will be recruited from the newly merged Mount Sinai-NYU Medical Center and Health Care System between Mount Sinai Medical Center and New York University Medical Center. This affiliation which started 7/1/98, encompasses the regions largest health care system, compromising 24 hospitals, 15 long-term care facilities and associated medical staffs and practices in New York City and surrounding environs. More specifically will be the role of the newly opened Barbara and Maurice Deane Prostate Health Center (PHC) at the Mount Sinai School of Medicine campus, under the direction of Dr Hall. The central focus of the Center will be a multi-disciplinary approach to diseases of the prostate with a special emphasis on exploring new therapies for patients with treatment failure, as the present proposal. There will be no competing protocol for patients in which radiation therapy has failed. Patients will be seen by a urologist, medical oncologist and a radiation oncologist under the same roof to provide broad scope care which will also include the services of a social worker, nutritionist, study coordinator and data manager. The Center will serve as the center of activities for the Departments close affiliation with the Bronx VA Medical Center and Elmhurst Hospital Center. The Department of Urology supports resident rotations at both

hospitals and staffs each site with faculty members. Members of the PHC staff at both sites can coordinate patient accrual.

Mount Sinai Hospital is located on the Southern edge of Harlem in New York City, serving a large Hispanic and African-American population. African-American males have a significantly higher incidence of prostate cancer than American white males and the highest mortality from prostate cancer in the world. Therefore, this population would especially benefit from new treatments for prostate cancer. Therefore, every step will be taken to include minority groups in this study. To this end, an important program of the Prostate Health Center is community outreach. This program, through a grass roots effort, aims to increase awareness and to educate the surrounding community about prostate cancer through a linkage of community health centers and churches. These efforts should enhance recruitment of patients for this trial.

Our Phase I trial of Ad.HSV-tk gene therapy for patients with locally advanced prostate cancer has accrued 6 patients. The racial breakdown is as follows:

Non-Hispanic White	Hispanic	Asian	African-American	Native American
2	2	1	1	0

Based on this breakdown we would anticipate accrual by race as follows for the expected 24 patients:

Non-Hispanic White	Hispanic	Asian	African-American	Native American
8	8	4	4	0

Inclusion Criteria:

Criteria which would be considered part of an evaluation for men with radiorecurrent prostate who are considering salvage therapy (radical prostatectomy, radiation seed implant, cryotherapy) or for men with hormone refractory disease as defined by a rising PSA following one secondary manipulation (addition or withdrawal of anti-androgen) as follows:

a. Men with recurrent prostate cancer after definitive treatment by radiation, either external beam or seed implantation or hormone refractory but non-bone metastatic disease. Failure is defined by a rising PSA on at least 3 occasions minimally each separated by 2 weeks and a prostate biopsy noting recurrent prostate cancer. Based on the age incidence of men with prostate cancer the age range would be expected to be 40- 80 years of age.

b. No evidence of metastasis by bone scan. Questionable areas on bone scan must be confirmed negative by plain films or MRI. Patients with hormone refractory disease may be included if they have lymph node metastases if confine to the pelvis by CT scan.

c. Patients must have adequate baseline organ function as assessed by the following laboratory values before initiating the protocol.

i. Adequate renal function with serum creatinine $\leq$1.5 mg/dl or creatinine clearance $\geq$45 ml/min/m^2.

ii. Platelet count $\geq$ 100,000 platelets/mm^3.

iii. Absolute neutrophil count $\geq$1000/mm^3.

iv. Hemoglobin $\geq$8.5 mg/dl

v. Normal partial thromboplastin time (PTT) and Pro-Thrombin Time (PT).

vi. Bilirubin $\leq$2.5 mg/dl, SGOT and SGPT <2.5x normal.

Criteria, which are set for the study only, are:

a. Karnofsky status $\geq$ 70%

b. Patients will agree to use condoms during any sexual activity for the duration of the study.

c. The patient will possess the ability to give informed consent and express a willingness to meet all the expected requirements of the protocol for the duration of the study. (Appendix A - Patient Consent Form).

Exclusion Criteria:

a. Patients who have taken immunosuppressive drugs within 2 months of study entry.

b. Acute infection. Acute infection is defined as any viral, bacterial or fungal infection, which requires specific therapy.

c. HIV positive tests.

d. Significant co-morbid medical or psychiatric conditions judged by the principle investigator to pose high risk for investigational study. Examples of psychiatric diagnoses, which would preclude participation, would include non-treated bipolar disorder, active psychosis, schizophrenia, or history of non-compliance with medications for such conditions.

III. Protocol Design

A) Patient Identification:

All patients will be identified with a unique number assigned to them at the time of registration to the trials. The number consists of the local IRB number followed by the sequential number of the patient in the trial (i.e., 01-0595-1, 01-0595-2, and 01-0595-03). This number is assigned to individual patient binders containing the case report forms for individual patients without any identification back to an individual patient. Binders are kept in the locked offices of the Prostate Health Center in a locked file cabinet. Access to the Office and file cabinet is restricted to the PI and study coordinator and physician assistant employed by the Prostate Health Center. The identity of a participant in the trial

will remain confidential with respect to any announcements or publications of the results of the trial. The Institution and the investigators will make efforts to provide protection from the media to protect privacy. The patient's medical record may be reviewed by representatives of applicable Federal agencies (FDA, OBA) and by the monitoring body of the School of Medicine.

B) Recruitment of Patients:

Patients will be recruited through the Prostate Health Center, which has a primary focus on patients with treatment failure. Patients will be drawn from several sources. First, a large number of patients would be screened from the practice of the Department of Urology, which over the years has had a significant role in the development of one of the largest Prostate Seed Implant programs in the world. Unfortunately, some patients do suffer from local disease recurrence and would thus be candidates for this trial. Because of the high profile status of this program many patient-directed referrals are seen for salvage radiation implants who would also be candidates for this particular study. Secondly, the Prostate Health Center has established an IRB approved e-mail-based mailing list of Urologists on staff at the Mount Sinai Medical Center for monthly postings of clinical trials open within the Center. This mailing consists of a cover letter outlining particular highlights and a short summary with inclusion/exclusion criteria for each approved trial. Thirdly, the Center has a website describing the mission and facilities within the Center. This information includes IRB approved layman's summaries of clinical trials such as the present protocol. Lastly, the Center serves as the center of activities for the Department's close affiliation with the Bronx VA Medical Center and Elmhurst Hospital Center to offer patients at those locations access to cutting edge treatments. Members of the Prostate Health Center also staff both locations and are intimately aware of clinical trials offered through the Center. Referrals would then be made to the Integrated Prostate Cancer Program within the Center. This particular endeavor is staffed by Dr Hall, medical oncologist and a radiation oncologist where patients are seen together with all the specialists in the same setting. This program is designed to provide expert opinion and care to patients with advanced prostate cancer, especially those patients with treatment failure. The process for recruitment would thus be coordinated through the Prostate Health Center. For approvals at these 2 sites, the IRB at Mount Sinai must first review and approve a protocol. At Elmhurst the Research Committee and at the Bronx VA Medical Center an IRB reviews the protocols.

C) Consent Process:

The consent process would entail a meeting with both the PI and Study coordinator. During the first visit information is provided through verbal explanations and some basic reading material, including the consent form, to both patient and family member. For interested parties a second visit would include further discussion with the PI of the study and a lengthy visit with the study coordinator. Sufficient time for the patient and his family to review the consent form in the home environment is provided to ensure that questions may be posed and answered and that the participant understands the protocol in detail. No patient will be consented during the first meeting. Prior to signing the consent it will be reviewed in detail by the PI and the patient and family if they are available in the presence of the Study Coordinator. The patient will then verbally, in his own words,

relay the major aspects of the trial. Once the consent has been signed the screening exams are performed, and arrangements are then made for the injection of Ad.hIL-12 and hospitalization in the GCRC. Based on prior experience there would be a minimum of 2-3 weeks before the screening process is complete and the vector injection due to time to complete the review of screening exams and practical issues of bed availability in the GCRC. Therefore, the process is designed to allow a significant amount of time to elapse between learning of the trial, the signing of the consent and the performance of the clinical trial.

For HIV testing a separate IRB approved consent designed for patients required to be tested for study purposes is used. Prior to signing the consent and blood drawing, the patient is counseled by the PI in a similar fashion for anyone undergoing HIV testing. The method of testing, the need for confirmation of an initial positive result by an ELISA test with a Western blot and the implications of a positive result reviewed. In the event of a positive result the patient would be counseled by the PI and referred to an Infectious Disease expert here at Mount Sinai. The assays are performed in the laboratory of the Medical Center with the initial assay performed by ELISA with confirmation of a positive ELISA via Western blot. The confidentiality of the patient will be maintained by sharing the results only with the patient and his primary care physician.

In an effort to understand the potential long-term effects of vector injection in tissues, permission is sought to obtain tissue samples after death. These tissues would be harvested during a requested autopsy with the specific interest other that of a routine autopsy, focusing on samples of the prostate and liver. Tissues will be studied histologically and be screened for expression of viral genes by PCR. This process is discussed at the initial entry to the trial. This will of course depend on the consent of the family to first perform an autopsy in a situation, which would not dictate the need and secondly obtain tissue samples. This will require a second consenting process with the next of kin. At the time of autopsy the pathologist will be asked to remove the prostate and a piece of liver. Each sample will be divided for formalin fixation and processing for histology and for freezing and RNA/DNA extraction.

D) Evaluation Prior to Treatment:

Once a patient has signed the consent the following parameters must provided to ensure that a patient meets the inclusion/exclusion criteria. Studies which would be considered standard of care for men with a rising PSA following definitive radiation who are candidates for salvage therapy (radical prostatectomy, salvage seed implant, cryotherapy) or who have a rising PSA while on hormone therapy for locally advanced prostate cancer are as follows:

a. A complete history and physical examination.
b. Blood Evaluation: CBC with differential & platelet count, PT, PTT, fibrinogen, fibrin split products, and D-dimers, Sma-6, creatinine, SGOT, SGPT, bilirubin, calcium, magnesium, phosphorus, PSA, HIV testing
d. Urinalysis and Urine culture.
e. Radiological Studies: CXR, Bone Scan, CT Scan Abd/pelvis
f. EKG

g. Prostate needle biopsy via TRUS probe to document recurrent prostate cancer.

Studies, which are to be performed as part of the research protocol, are as follows:

a. serum titer antibodies to adenovirus, and serum for ELISA for cytokines (IL-6, IL-12, IFN-γ, TNF–α)

b. Microbiology studies: Presence of adenovirus in serum and urine

c. Peripheral blood lymphocytes for T cell assays on 3 separate occasions

E) Cohort design:

The initial dose of Ad.hIL-12 will be set 4 logs lower than the therapeutic dose used in the pre-clinical studies on the basis of weight correlation between mice and humans. A total of 3 patients will be enrolled within each cohort and be evaluated for toxicity as a minimum response criteria. If an individual patient is not available for toxicity evaluation, an additional patient will be enrolled to ensure a 3-person cohort for each dose. A total of 24 patients are expected to be enrolled.

	Plaque forming units (pfu)	vector particles (vp)
Cohort 1	$1x10^9$	$6x10^9$
Cohort 2	$3.3x10^9$	$1.98x10^{10}$
Cohort 3	$1x10^{10}$	$6x10^{10}$
Cohort 4	$3.3x10^{10}$	$1.98x10^{11}$
Cohort 5	$1x10^{11}$	$6x10^{11}$
Cohort 6	$2x10^{11}$	$1.2x10^{12}$
Cohort 7	$3x10^{11}$	$1.98x10^{12}$

The starting dose of $1x10^9$ pfu is over 4 logs lower than the MTD/therapeutic dose used in mice, as calculated through comparison of average body weight of a mouse (30gm) and a man (75Kg). For the first 5 cohorts dose escalation will be by half-log increments. The last 2 cohort escalations are arithmetic rather than logarithmic on advice from the FDA over concerns for potential toxicities at higher doses. Dose escalation to the next dose cohort may be occur only after a 30 day waiting period following injection of the last patient in the lower dose cohort and a thorough review of toxicity data within that dose.

This study is a classic 3 patient cohort dose escalation. If no DLT is observed in 3 patients at a cohort dose level, the vector dose will be escalated to the next dose. If DLT is experienced in 1 of 3 patients at a given dose, an additional 3 patients will be treated at the same dose. If DLT is observed in any of the additional patients, enrollment will cease at that level and no further escalation will continue. The previous dose cohort will be expanded with an additional 3 patients. If no DLT is observed in the additional 3 patients, dose escalation to the next cohort will continue. If DLT is observed in 2 patients within the previous dose cohort, then no further patients will be enrolled at that dose. The maximum tolerated dose (MTD) will be defined as the highest cohort level at which less than 2 instances of DLT are experienced in 6 patients. Once this is reached, the FDA will be informed. If DLT is not seen at the highest dose cohort ($2.85\ x10^{13}$vp), the protocol will be amended at that time to include further dose escalations.

F) Injection Procedure:

Patients will be admitted to the GCRC at the Mount Sinai School of Medicine on the morning of vector injection. Patients will transported to the Cystoscopy Suite for the procedure. Since present day imaging is extremely poor at accurately assessing the extent and localization of cancer within the prostate, the dose of virus will be divided into 10 aliquots, each in 500uls, to be diluted with phosphate buffered saline as needed. To ease the potential for discomfort related to the procedure, a prostate nerve block will be performed. This is achieved through using a Transrectal Ultrasound (TRUS) guided injection of local anesthetic via a standard protocol used during prostate biopsy. Specifically, 4mls of 1% lidocaine is injected on both sides of the base of the prostate where the seminal vesicle attaches to the prostate gland. After waiting for several minutes the Ad.hIL-12 will be injected at 5 sites on each side of the prostate (right and left), extending from the base to the apex, for a total of 10 injections. Injections will be performed under real time ultrasonography via a 21 gauge needle. The injection needles will be placed sequentially in a pattern to best expose as much of the tumor as possible to the adenoviral vector. After each vector injection the needle is withdrawn and replaced into the next tumor area for the next aliquot of virus injection and so on. Total volume to be injected will be 5mls. Vital signs will be monitored during the procedure.

After injection the vial containing the vector, injection needle and syringe are cleaned in bleach prior to disposal in marked red bags, as per Institutional policy. Any unused vector is returned to the Vector Core facility. In the event of accidental spills of the virus from the vial, common disinfectants can be used to inactivate the virus. All personnel are instructed on safe use and potential biological hazards of the virus vector before the procedures are performed.

G) Evaluation Following Ad.hIL-12 Injection

After the procedure, the patient will be transported back to a private room in the GCRC with private bathroom facilities. An individual patient may only share a room and bathroom facilities with another patient injected with Ad.hIL-12. Each patient will remain hospitalized until the cytokine levels return to baseline Patients are expected to be hospitalized for 10-14 days post-vector injection.

a. Physical examination: Examination will be performed on days 2,3,4,5,6,7,8,9,10, 14, 28, 42, and 56 post-vector injection.

b. Blood Studies:

-CBC with differential and platelets, electrolytes, BUN, creatinine, and liver function tests, on days 2,3,4,5,6,7,8,11,14, 28, 42, and 56 post-vector injection.

-PT and PTT on days 2,4,6,8,14,28,42,and 56 post-vector injection

-Fibrinogen, fibrin split products and D-dimers on days 2, 4, 6, & 8 post-vector injection.

-Adenovirus in serum & urine days 1, 7, & 14.

-PSA days 7, 14, 28, 42 and 56 post-vector injection.

-Serum IL-6, IL-12, IFN-γ and TNF-α on days 2,3,4,5,6,8,11,14 post-vector injection. Blood draws will continue every 3 days after 2 weeks until levels of any cytokine have returned to baseline.

The total blood volume per day for these assays will be 30mls (~2 tablespoons).

- Serum neutralizing antibodies to adenovirus days 7, 14, 28 post-vector injection. The will be an extra 5 mls of blood (~1 teaspoon).

-T cell assays with PBMCs on days 14, 28,and 56. This will be achieved by drawing an extra 60 mls of blood (~4 tablespoons).

Blood tubes for standard and special chemistry items are labeled with an imprint of an individual patient's hospital card. Blood for ELISA measurements and T cell assays (ELISPOT) will be labeled with an individual patients study number (01-0595-1, 01-0595-02 etc) and stored in a –80^{0}C freezer in Dr. Chen's laboratory. Access to the freezer is restricted to members of her lab by lock and key.

After day 8, for any grade $\geq$2 laboratory abnormality, the test will be repeated daily until the results return to grade 1 status. After day 8, any grade 1 abnormality will be repeated biweekly until the abnormality has resolved. Post-treatment tests after 14 days through 56 days may be performed within a 3-day window period. Patients will be followed exclusively by the Prostate Health Center for a period of 3 months after which patients will be followed either by the Prostate Health Center or the referring physician with follow up contact concerning treatment outcome, and survival made by telephone. The 3 month period represents the time for monitoring for serious adverse or unexpected events and the time period after which a continuing rise in PSA would be considered failure of therapy and such patients would be released for further therapeutic options. To ensure that patients follow up appropriately after discharge, individualized calendars outlining future appointments will be created for each patient immediately after vector injection. Each appointment will be placed on the computerized scheduling service (Outlook) used for all patients in the PHC, to thereby ensure review of upcoming appointments by the PI and study support personnel. Telephone and letter will follow up missed appointments. These deviations will also be noted in the medical record.

IV Risks & Benefits

Risks of Screening Process

A) Potential Complications of Prostate Needle Biopsy with Local Anesthesia

Infection: Prostatic and urinary tract infections and systemic sepsis are possible because of the transrectal route of administration, but occurs in less than 1% of patients who undergo prostate biopsy. Antibiotic therapy will be given as prophylaxis prior to and after injection of the virus into the prostate.

Hematuria: Gross hematuria following prostate biopsy occurs in 25% of patients and is usually self-limited. If severe, <1%, a Foley catheter may need to be placed until the bleeding stops.

Urinary retention: Injection of virus into the prostate may cause local swelling which could lead to some change in urinary habits and in the worst case scenario, acute urinary retention. A Foley catheter will be placed if needed.

Pain: Some patients experience transient pain after a prostate needle biopsy requiring analgesics

B) Potential Complications of Screening X-ray Studies.

The pelvic CT scan will be performed with IV contrast. There is a small risk (<1%) of an allergic reaction to the contrast material, manifest in mild cases by a rash and anaphylaxis at its worst. This potential complication is minimized by excluding patients with a history of contrast reactions and allergies to shellfish. Patients are carefully monitored during the procedure to identify any problems as early as possible. Contrast may also result in renal failure in less than 1% of patients, usually only transiently. Higher risk patients, those with serum creatinine >1.5mg/dl will be excluded from having contrast, while diabetic patients are generally hydrated during the procedure to avoid this complication.

C) Potential Complications of Intravenous Procedures

During the screening and hospitalization patients will have a variety of either intravenous injections (CT Scan, bone scan) or blood draws which in addition to pain can result in an infection. Using alcohol to clean the skin and single use needles and catheters for procedures minimizes this complication.

Risks of Ad.hIL-12 Therapy

A)Potential Complication of Virus Injection

Infection: Prostatic and urinary tract infections and systemic sepsis are possible because of the transrectal route of administration, but occurs in less than 1% of patients who undergo prostate biopsy. Antibiotic therapy will be given as prophylaxis prior to and after injection of the virus into the prostate.

Hematuria: Gross hematuria following prostate biopsy occurs in <1% of patients and is usually self-limited. If severe, a Foley catheter may need to be placed until the bleeding stops.

Urinary retention: Injection of virus into the prostate may cause local swelling which could lead to some change in urinary habits and in the worst case scenario, acute urinary retention. A Foley catheter will be placed if needed.

Pain: Some patients experience transient pain after a prostate needle biopsy requiring analgesics. Since vector injection is performed with a 21-gauge needle in contrast to an 18-gauge core needle used for biopsies, pain should be easily controlled.

B) Potential Complications Specific to Ad.h IL -12 Injection

The complications of intra-prostatic injection of Ad.hIL-12 are unknown.

Intravenous and subcutaneous administration of recombinant IL-12 has been evaluated in Phase I clinical trials (1,2). Dose limiting toxicities include stomatitis and liver dysfunction as manifest by elevated serum aminotransferases. Other effects noted were fevers, chills, nausea/vomiting, anemia, thrombocytopenia, leukopenia and headache.

Intratumoral injection of mIL-12 protein, Ad.mIL-12 and autologous/allogeneic fibroblasts transduced with a retroviral vector expressing mIL-12 has been studied in mice. Subcutaneous renal cell cancer implants injected with autologous fibroblasts expressing mIL-12 show that at therapeutic doses, the treatment was well tolerated without toxicity manifest in measurements of renal and liver function and no

pathological changes noted in liver, spleen or lung (3). Likewise, injection of Ad.mIL-12 into liver tumors of colonic metastases at therapeutic doses noted only transient minimal-moderate elevations of transaminases (4). Experience with the orthotopic mouse model of prostate cancer mirrored this experience with all mice remaining healthy during after injection of Ad.mIL-12. Preliminary results of Phase I clinical trials with vaccination strategies of either transduced fibroblasts or tumor cells notes immune responses without concomitant serious toxicities.

Further concern may arise over the dissemination of vector within the body, both to vital organs and within the genitourinary tract and the potential for exposure to germ cells. Pre-clinical studies in mice explored this question following injection into the dorsolateral prostate with an adenovirus expressing Herpes Simplex Virus thymidine kinase gene (HSV-tk). Spread of the vector to adjacent structures was evaluated by PCR (5). One week following injection vector sequences were detected at the site of injection at a level of 1 copy per cell or less. Vector was also detected in the adjacent ventral prostate at levels over one hundred fold lower than in the dorsolateral prostate. In the seminal vesicle 45% of the animals injected had detectable vector sequences at levels at levels less than 0.1 copy per cell but no vector was detected in the seminal fluid. Spread of vector sequence top the testis was noted in 1 of 28 mice. However, examination of sperm harvested from the epididymis of injected mice failed to detect any vector sequences. Further, more direct studies evaluated the potential for germ-line transmission of an adenovirus expressing β-galactosidase (Ad.β-Gal). Initial studies addressed what cells are infected following injection into the testis or epididymis (6). Beta-galactosidase expression was only noted within the interstitium - Leydig Cells - with the complete lack of expression within the germinal epithelium. Furthermore, exposure of sperm to Ad.β-Gal reduced the ability of successful IVF and examination of resulting embryos failed to reveal any β-galactosidase. Therefore, it appears that retrograde spread of Ad.mIL-12 into the testis could potentially occur at a low incidence but is unable to transduce individual sperm.

Vector spread to other organs was also tested following intraprostatic spread of Ad.HSV-tk. Again by PCR screening detected spread of virus 7 days after vector injection to the liver in less than 10% of the animals (5). Spread to the gut and lung was not detected. No obvious pathology was observed on microscopic examination in any vital organ.

It is theoretically possible that recombination with a latent wildtype adenovirus harbored by the patient could reintroduce the E1a region back into the virus in exchange for hIL-12. Such a recombinant virus that contained the RSV and/or IL-12 genes and the regained E1 sequence would be too large for packaging in the viral protein coat and would not be recoverable. No such recombination has been detected in animals or 293 cells. A recombinant virus that expresses E1 would resemble a wild type virus that would cause mild, self-limited infection.

Potential Biohazard Exposure to Health Care Personnel and Others:

Personnel involved in virus injection: There is a low potential for biohazard exposure to the personnel involved in virus injection. The viral vector is suspended in a small volume of buffer and is contained in a septum vial. The syringe is loaded from the vial

with no virus vector loss. Intratumoral injection is made and the amount of leakage along the needle track is likely to be minute. In the event of accidental spills of the virus from the vial common disinfectants can be used to inactivate the agent. All personnel are instructed on the safe use and potential biological hazards of the virus before the procedures are performed.

Post surgical health care workers: There is a potential that Ad.hIL-12 may escape the patient via urine feces, saliva, mucus, tears or other excretions. Studies in non-human primates indicate that that potential is low. Studies show that only one plaque was detected in the serum of one baboon of six that were injected two days previously with the vector and minimal virus was detected in semi-permissive host, cotton rats after intracardiac injection (FDA IND#6371). Exposure to shedded replication defective virus will pose a very limited hazard, as the titer will be extremely low. There is a theoretical possibility that the vector could recombine with wild type adenovirus harbored by the patient (see above). Infection with this virus would cause a mild self- limited infection similar to a common adenovirus infection. Health care workers will be instructed on the safe use and potential biological hazards before they are allowed to work with patients. Standard body fluid precautions will be enforced.

After injection of Ad.hIL-12 patients are admitted to a single or double room in the GCRC. Patients can only share another room with another patient treated with Ad.hIL-12. Patients may have visitors who must be instructed in and follow standard body fluid precautions. Each patient is asked to wear a condom during sexual activity for the duration of the study.

Benefits of Ad.hIL-12 Therapy

As a Phase I Clinical trial no definitive statements can be made concerning therapeutic effects to a patient. It states clearly in the consent form that while data from animals notes the ability of a single injection of Ad.mIL-12 virus to control growth of both the injected tumor and pre-existing metastases through a variety of mechanisms, there is no evidence that this will occur in patients. That said, the population chosen for study, clinically localized radiorecurrent prostate cancer, have few viable options for further therapy. Within the guidelines laid for the trial patients will not be risking harm of disease progression by enrolling in this trial and will still have other options available to them after participating in this study.

V Reporting of Serious or Unexpected Adverse Events and Toxicity

Serious or Unexpected Adverse Events

A serious adverse event as defined by the FDA is any adverse experience ensuing from participation in this trial which results in death, injury, prolongs or leads to hospitalization, and/or necessitates medical care of a participant acutely or for a chronic disability. An unexpected adverse event is defined as an adverse experience, which is not listed as a potential risk in the consent or is more severe than that indicated by the investigators within the protocol or consent. A serious adverse event, both expected and unexpected, must be reported, regardless of whether the PI feels that the event was

related to participation in the clinical trial, to the Mount Sinai IRB, FDA, Office of Biotechnology Activities (OBA) and the USAMRMC. Initial contact for each agency is via the telephone, followed by a written report within 3 days. Reports to the FDA are via form 3500 (Medwatch - Attached) and to OBA via a prescribed format for Human Gene Transfer Studies (Attached). The agencies and addresses are as follows:

Dr. Jeffrey H. Silverstein	OBA	FDA, Off of Therapeutics Research
Chair, IRB	NIH, MSC 7010	Ctr.forBiologicsEvaluation& Research
One Gustave L. Levy Place,	6705 Rockledge Dr	HFM-99, Rm 200N
Box 1075	Suite 750 MSC 7985	1401 Rockville Pike
Mount Sinai School of Medicine	Bethesda, MD 20892	Rockville, MD20852-11448
New York, NY 10029	(301)496-9838	
(212)659-8980		

USAMRMC
ATTN: MCMR-RCQ
504 Scott St
Fort Detrick, MD 21702-5012
(301)-619-2165.

Toxicity Grading and Reporting

Toxicity will be graded on a 0-4 scale according to common toxicity criteria from NCI (Appendix III). All adverse events will be reported. Reporting requirements depend on the severity and unexpectedness of the toxicity encountered.

	Grade I	Grade 2	Grade 3	Grade 4	Grade 5
Phase I					
Expected	Flow sheets	Flow sheets	Flow sheets	Phone/written	Phone/written
Unexpected	Phone/written	Phone/written	Phone/written	Phone/written	Phone/written

Measurable parameters for the assessment of hepatic toxicity are as per CTC

	Normal Range	Grade1	Grade 2	Grade 3	Grade 4
Serum ALT (U/L)	0-50	>50-125	>125-250	>250-1000	>1000
Serum AST (U/L)	0-53	>53-133	>133-265	>265-1060	>1060
Serum bilirubin (mg/dl)	0.1-1.2	>1.2-3.0	>3.0-6.0	>6.0-24.0	>24.0

The DCT Reaction form for Investigational Drugs will be used for reporting such events. Phone communication with the study chairs will be made within 24 hours and the written report within 5 days. The IRB, the FDA and the Office of Recombinant DNA Activities will be notified of all adverse events, serious events and unexpected events as per existing policies.

In study patients where elevations in serum cytokines are encountered in the absence of clinical toxicity, threshold levels have been mandated, above which an elevated serum

level will be considered a dose limiting toxicity. The exact levels have been determined from clinical trial experience with recombinant products (IL-12, IL-6 & TNF-α and represent the MDT's in those trials. Threshold levels will be as follows:

IL-12: 11,000 pg./ml	IFN-γ: 6000 pg./ml
IL-6: 4000 pg./ml	TNF-α: 12000 pg./ml

Dose limiting toxicity (DLT) is defined as any grade ≥3 toxicity, including hematological toxicity and constitutional symptoms. If no DLT is observed in 3 patients at a cohort dose level, the vector dose will be escalated to the next dose. If DLT is experienced in 1 of 3 patients at a given dose, an additional 3 patients will be treated at the same dose. If DLT is observed in any of the additional patients, enrollment will cease at that level and no further escalation will continue. If no DLT is observed in the additional 3 patients, dose escalation to the next cohort will continue. If DLT is observed in 2 patients within a cohort, then no further patients will be enrolled at that dose. If DLT is not seen at the highest dose cohort ($2.85x10^{13}$ vp), the protocol will be amended at that time to include further dose escalations. The maximum tolerated dose (MTD) will be defined as the highest cohort level at which less than 2 instances of DLT are experienced in 6 patients.

Dose escalation to the next dose cohort may be occur only after a 30 day waiting period following injection of the last patient in the lower dose cohort and a thorough review of toxicity data within that dose.

Depending on the toxicity encountered a minimum of 24 patients will be enrolled in this trial.

VI: Description of Ad.hIL-12 Vector and Other Medications

The recombinant adenoviral vector Ad.RSV-hIL-12 has been constructed and characterized as described (7). Specifically, this is a replication incompetent E1-deleted adenovirus that contains the p40 and p35 IL-12 subunits, connected by an internal ribosomal entry site, were placed under the control of the Rous Sarcoma virus promoter. The seed viral vector and large-scale vector production will be produced under GMP conditions in Vector Production Core facility at the Mount Sinai School of Medicine under the direction of Dr. Savio Woo.

A) Preparation of the seed vector:

The functional recombinant human IL-12 virus will be purified by one round of re-plaquing and amplified by inoculating onto 293 cells seeded in 4ml of medium in 60mm plates. When cytopathic effect is present throughout the plate, the cells and conditioned media will be harvested, divided into aliquots and kept frozen at -70^{o}C. This material will be the seed virus for testing and for the subsequent production of the vector.

B) Large-scale vector production:

GMP production will proceed following demonstration of functionality of the amplified material. The equivalent of at least one hundred 15cm plates, seeded with 293

cells from a fully characterized cell bank, will be used for each production run. Following demonstration of CPE, the cells will be harvested by centrifugation and the cell pellet will be resuspended in condition medium. The concentrated cell will be subjected to three rounds of freeze/thaw and the cellular debris will be removed by centrifugation at low speed. The clarified lysate will be purified by one round of cesium chloride (CsCL0 step gradient ultracentrifugation (1.40g/ml and 1.25g/ml, 35,000rpmx1hr at 4^0C). The lower band containing infectious particles (defective particles are lower in density and form a higher band) will be harvested, diluted, and dialyzed against four changes of dialysis buffer (100X volume at 4^0Cx1hr), filtrated through a 0.2um filter at the end of each run. The total particle concentration is determined by measuring the OD260 and the filtered virus preparation is aliquot into vials labeled with vector designation, lot number, particle concentration, volume, preparation date, and storage condition. The filled vials are stored at -70^0C. The recombinant adenoviral vector will also be quantified by determining the infectious particle concentration by plaque assay.

C) Testing of the master seed stock and production lots:

All testing required for release of clinical grade material will be contracted to a FDA approved independent testing facility that has properly validated assays in place. Tests will be performed to detect mycoplasma, adventitious viral contaminants, AAV, sterility, endotoxin, replication competent adenovirus, as well as cesium chloride concentration and determination of functionality. The FDA also requires sequencing the entire adenovirus vector. Currently our Core facility has all the adenovirus primers and demonstrated this practice to sequencing entire adenovirus genome. The functionality assays which include the ELISA, IFN-γ induction assay and induction of NK activity as previously described (4).

D) Storage and release of Ad.hIL-12.

Aliquots of vector are stored in a locked -70^0C freezer under the auspices of the Vector Core Production Core Facility. Release of vector to each PI begins with review of IRB, RAC, FDA and DOD approval documents, a copy of which are kept on file in the Core Facility. Between each dose cohort release of vector for the next dose is dependent on favorable review of reports from the Medical monitor and regulatory monitor. Unused drug will be stored under the auspices of the Vector Core in a locked freezer. Since it is GMP quality it could be used in other approved clinical trials.

E) Other Medications

a. Antibiotics: All patients will receive a broad spectrum oral antibiotic the evening before and the morning of the procedure, continuing the antibiotic twice daily for a total of 5 days.

b. Granulocyte colony stimulating factor (G-CSF): Patients may receive G-CSF support for neutropenia (<500 cells/mm^3).

VII Flow of Information, Records to be Kept and Trial Monitoring.

When a patient enters the study registration forms will be forwarded to the Data Manager. A Study File containing the patients' name, patient number, unit number, location, attending physician, age, sex, and diagnosis will be created. A study number will be issued for each patient, based on the study number generated by the IRB and the number of the sequential number of the patient enrolled. Four copies and the original signed consent form will be filed. The original will remain in the hospital chart with duplicates going to the patient, protocol chart and office chart. A protocol chart containing registration form, consent, eligibility chart, disease status data, history and physical forms, and other appropriate physician's notes will be created. NCI designed flow sheets will be utilized for the initial evaluation and day to day observations, vital signs, and laboratory results. A protocol-specific sheet has been designed for documenting the Protocol charts are kept within the research office of the Deane Prostate Health Center in a locked file cabinet. Access to this locked office is restricted to the Administrator of the Department of Urology and the personnel of the Center (Hall and Knauer).

As per the regulations of the governing bodies, patient's charts may be reviewed by the Medical Monitor, Regulatory compliance officer identified by the IRB, the staff of the IRB, and representatives of the Federal Agencies, FDA and OBA. In addition representatives of the US Army Medical Research and Material Command are eligible to review research records as part of their responsibility to protect human subjects in research.

The IRB of the Mount Sinai School of Medicine has stipulated the need for 2 types of monitoring for clinical gene transfer studies: data/patient safety monitoring and regulatory monitoring. Since the PI of the study also serves as the FDA Sponsor, reports of monitoring are directed to him, the IRB and IBC. Data and patient safety monitoring will be achieved through a Data and Safety Monitoring Board (DSMB) consisting of experienced investigators at Mount Sinai. Prior to the initiation of the study the list of names of members for the DSMB is presented to the IRB for approval. Regulatory monitoring is provided by a trained individual employed by the Office of Clinical Trials at the Mount Sinai School of Medicine. The Monitor is a physician and is also responsible for reviewing SAE including forwarding her report to the DOD as stipulated. Monitoring is performed each 6 weeks or sooner if a 3 dose cohort is completed. Generated reports are forwarded to the IRB, IBC and the Sponsor/PI and must be reviewed prior to initiation of the next dose cohort.

VIII Protocol Modifications or Departures

As per the regulations of the organizations involved in this study, any change in the protocol must be filed with and where indicated approved prior to instituting said change. In the event of the need to alter the protocol or consent, the revised paperwork will be forwarded to the Mount Sinai IRB, the FDA, OBA and HSRRB for review and approval. Likewise, any deviation from the protocol must be documented in the medical record and the information forwarded in writing to the Mount Sinai IRB, the FDA, OBA and HSRRB. The origin of such deviations would be expected to come to light through

reviews by the PI & study coordinator, Medical monitor and the DSMB. In each case missing data points would come to light through a review of source and trial documents and the trial documents.

IX Roles & Responsibilities of Study Personnel.

Simon J. Hall, MD: PI and Sponsor. Dr, Hall in his capacity as both PI and sponsor of this trial assumes the responsibility of overseeing the entirety of the study, from screening potential patients to regulatory review. He will be specifically responsible for consenting patients, the injection procedure and providing patient care during the planned hospitalization.

Shu Hsia Chen Ph.D.: Co-Investigator. Dr Chen is responsible for overseeing the performance of the ELISA measurements of cytokines and the assays examining T cell activity following Ad.hIL-12 treatment. These assays are performed in her laboratory.

John Mandeli, Ph.D.: Co-Investigator. Dr Mandeli was instrumental in the statistical design of the trial and will be performing statistical analysis on data from the trial.

Cynthia Knauer, RN, MS: Research Study Coordinator for the PHC. Ms Knauer will responsible for screening potential patients, coordinating study and monitoring visits and maintaining the study records.

Michael J. Droller, MD: Medical Monitor. Dr. Droller is a qualified physician, other than the Principal Investigator, who is not associated with this particular protocol, who is able to provide medical care to research subjects for conditions that may arise during the conduct of the study, and who will monitor the subjects during the conduct of the study. The medical monitor is required to review all serious and unexpected adverse events associated with the protocol and provide an unbiased written report of the event within ten (10) calendar days of the initial report. At a minimum the medical monitor should comment on the outcomes of the adverse event (AE) and relationship of the AE to the study. The medical monitor should also indicate whether he/she concurs with the details of the adverse event report provided by the study investigator.

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Appendix I: Cytokine & Immune Assays

Cytokine Assays:

Many lessons have been learned from the death of a teenager in a Phase I clinical trial of adenovirus gene therapy. While the events underlying the death of this patient remain unclear, it appears that some problems may have been due to an inflammatory response against the vector following intra-arterial infusion. Therefore, the RAC had mandated careful monitoring of pro-inflammatory cytokines even in instances of intra-tumoral injection of vector. Serum will be analyzed on days 2,3,4,5,6,8,11,14 post-vector injection. Blood draws will continue every 3 days after 2 weeks until levels of any cytokine have not returned to baseline. Much like IL-12, threshold levels of each cytokine have been adopted: IFN-γ - 6000 pg./ml; IL-6 - 4000 pg./ml; and TNF-α - 12000 pg./ml. Even in the absence of clinical manifestation of other toxicities, cytokine levels above these thresholds will be considered a dose-limiting toxicity. As with IL-12 patients will remain in the GCRC under close monitoring until such time that all cytokine elevations have returned to normal.

Statistical Consideration: To monitor changes in serum levels of the pro-inflammatory cytokines, blood will be obtained prior to vector injection in addition to post-injection to be used as a baseline for each cytokine. Data will be graphed for each patient over time. Peak levels will be obtained for each patient and will be summarized for each dose cohort by calculating the median peak serum cytokine level (and range of peak levels). The day the peak level occurs and the time for return to baseline levels will also be noted. In addition to analyses on serum cytokine peak levels, repeated measures analysis of covariance, with baseline serum cytokine levels as covariates, will be utilized to compare dose level groups with respect to serum cytokine levels over all time points. Repeated measures analysis of covariance will allow testing for a dose level group effect, a time effect, and a dose level group by time interaction. Before proceeding with repeated measures analyses, a sphericity test will be performed to check assumptions concerning the structure of the covariance matrix. If the sphericity test is significant, the Huynh-Feldt adjustment to F tests will be implemented. However, if the sphericity test is highly significant, repeated measures analysis of variance will be abandoned in favor of a mixed model analytic approach.

Immune Assays:

As outlined earlier, mechanistic studies revealed 2 chronologically distinct growth suppressive activities within the primary tumor. First, within 24-48 hours of Ad.mIL-12 injection discrete areas of necrosis associated with high levels of apoptosis and PMNs infiltration are noted. Second, beginning ~ one week after injection both a NK and T cell response are seen associated with a second peak of both IL-12 and IFN-γ. Together, these factors are important in mediating growth responses of a rapidly growing primary tumor. However, in patients to be treated both the primary tumor and any microscopic metastases will be are growing significantly slower thereby maximizing potential

efficacy. In Phase I clinical trials the FDA has set some rules governing allocation of tissue after therapy. Therefore, correlative studies have been set up to maximize accumulation of data to mirror that of the pre-clinical model, while abiding by FDA advice and patient comfort/safety. Therefore, tissue harvesting for analysis of apoptosis, PMN infiltration and changes in Fas expression will be deferred until a MTD is achieved, expected to occur outside the time line of this proposal. Due to the re-negotiation of the original budget, the NK assays have been dropped from the original proposal to leave monies for the toxicity studies and the measurement of T cell activity.

Measurement of T cell Responses

Isolation of T and DCs: PBMC will be isolated using standard methodology routinely used in our laboratory. Lymphocytes will be cryopreserved in medium containing 25% human serum and 10% DMSO. T cells will be obtained using positive selection with magnetic beads (Mini MACS Miltenyi Biotec). Briefly, cells are mixed with Microbeads, mixed well and incubated for 15 minutes at 4^{o} C. After washing, cells are resuspended in buffer. A positive selection column is placed in the magnetic field of a magnetic cell separator on a MACS multi-stand with a centrifuge tube under the column for collection. The cells are applied to the column. The negative cells pass through and the effluent is collected in the centrifuge tube. The column is placed over a new 15-ml centrifuge tube. One ml of buffer is pipetted onto the column, the positive fraction is flushed out using plunger supplied with the column, and the cells are counted. Dendritic cells (DC) will be prepared from (1,2). Previously frozen PBMCs will be plated on plastic and after 2 hours the non-adherent cells will be removed. Medium containing 1000 U/ml of IL-4 and GM-CSF each will be added, half of which will be changed every 2-3 days. After 7 days, these immature DC will be matured by adding TNF-α (5-10ng/ml).

Monitoring of Prostate Specific T cells: T cell mediated responses will be evaluated based upon IFN-γ and IL-10 secretion measured using the ELISPOT assay. IFN-γ were chosen since 1) IFN-γ is a product of cytotoxic cells and 2) IFN-γ and IL-10 are Th1 and Th2 cytokines, respectively, and thus may be informative concerning the mechanisms underlying any responses observed. Three pre-treatment blood samples will be taken to serve as baseline values. After Ad.hIL-12 injection, bloods will be drawn on days 14, 28, and 56. The ELISPOT assay will be carried out as previously described (3). Prior to testing in the ELISPOT assay, T cells isolated from using positive selection with magnetic beads (Miltenyi Biotech) will be presensitized in vitro against autologous DCs pulsed with antigen to increase the frequency of specific responding cells to detectable levels. DCs will be generated from T cell-depleted by stimulation for 7 days in medium containing 1000 U/ml of GM- CSF, 1000 U/ml of IL-4 and 1% human serum. These immature DCs will be matured by culture with 5ng/ml TNF- . Antigens used to pulse the will be 0.5 - 2.0 ug/ml PSA (Chemicon Int., Temucula, CA), 0.5 - 2.0 ug/ml PAP (Chemicon), or ovalbumin 2.0 ug/ml (Sigma Chemical Co., St. Louis, MO) as a negative control. PHA will serve as a positive control.

After 6 days, pre-sensitized cells ($0.2\text{-}0.5x10^{6}$) will be stimulated in microtiter plates with autologous DC pulsed either with the sensitizing antigen, or with flu,

unrelated melanoma peptides or unpulsed APC, the latter three serving as specificity or negative controls. Recombinant cytokine and medium will serve as positive and negative technical controls for the assay, respectively. Spots will be read with an automated ELISPOT reader (Zeiss) The number of T cells that respond will be calculated by subtracting control values (T cells stimulated with unpulsed APC). Results will be expressed as the number of responding cells per 10^5. Groups will be compared by the two-sample t-test for independent samples (unpaired t-test) with equal or unequal variances. The t-test for unequal variances will be used if the variances are significantly different by an F test. All tests will be two-sided.

$CD8^+$T cells from HLA-0201^+(A2) patients who give positive responses to prostate antigens after treatment compared to before treatment will be tested a second time in ELISPOT using defined PSA and PSMA peptides. The PSA peptides used will be PSA-1 (FLTPKKLQCV) and PSA 3 (VISNDVCAQV) (4); PSMA peptides will be PSMP1 (LLHETDSAV), PSM-P2– (ALFDIESKV) (5). This will enable the enumeration of the frequency of $CD8^+$ cells, as well as identify the epitopes recognized.

Statistical Considerations: Treatment effects for individual patients will be measured as paired differences pre- and post-therapy in measurements of chromium release at various times. Paired t-tests will be used for this analysis. Transformation of the data will be performed if appropriate, e.g. log transformation and hence treatment effect will be expressed on a log scale.

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Appendix II: Background

Prostate cancer remains the most commonly diagnosed organ cancer in US males, estimated at 180,000 diagnoses and 32,000 deaths in the year 2000 (1). Patients diagnosed with clinically localized cancer may chose as definitive therapy radical surgery or radiation therapy. Since its inception at the turn of the 20^{th} century, radiation therapy has progressed to several treatment approaches, including conventional external beam therapy, seed implantation (brachytherapy) and conformal 3-dimensional radiation therapy. Use of serum prostate specific antigen (PSA) levels as an indication of the continued presence of prostate cancer have allowed for a sensitive determination of treatment success or failure significantly earlier than could be achieved by clinical exam (digital rectal exam for local failure or bone scan for metastatic disease). Furthermore, PSA levels along with clinical stage and Gleason score at the time of initial treatment have been useful in stratifying patients into high risk, moderate risk and low risk patients. With conventional radiation therapy the 5 year recurrence rates for patients with clinically localized cancer (T1/T2) treated with external beam radiation therapy (XRT) range from 30% to 60% (2-5) depending on initial clinical stage, gleason score and PSA level. Patients with PSAs >10 have an ~50% chance of treatment failure (6). Likewise, patients undergoing brachytherapy with I^{125} seeds who have PSAs >10 or Gleason score $\geq$7 have 41% or >50% risk, respectively, of failing therapy within 4 years (7). Approaches to improve on these results have either increased radiation dose through conformal therapy alone or adding a supplementary seed implantation and/or the addition of hormone therapy. Early studies have indicated improved results but significant numbers (~30%) of patients suffer disease recurrence within 5years (6,7); to date, the longer-term efficacy of these newer therapies is unclear.

Treatment options for patients with radiorecurrent prostate cancer are limited both in terms of long-term disease free success and severe morbidity impacting on quality of life. In general patients undergoing salvage prostatectomy are highly selected - never-the-less 60% of patients have extraprostatic disease at the time of surgery, ~20% have lymph node metastases and only 44% are progression free at 10 years (8). In addition, salvage radical prostatectomy has been associated the significant morbidities of incontinence (>50%) and impotence (essentially 100%). Other options for local therapy in radiorecurrent prostate cancer include salvage brachytherapy and cryotherapy. The ability of these therapies to provide long-term disease free survival are unproven (brachytherapy and cryotherapy) and may be associated with morbidity, as in the case of cryotherapy of severe incontinence and recalcitrant penile/perineal pain. Therefore, the majority of patients with radiation recurrent prostate cancer will ultimately be treated with hormone ablation, a non-curative option associated with significant quality of life issues including hot-flashes, loss of libido, lack of energy and depression. Therefore, new treatment strategies for patients with radiorecurrent prostate cancer are needed: such an approach would ideally have limited morbidity and demonstrate both local and systemic anti-cancer characteristics.

While the widespread use of PSA has resulted in more patients being diagnosed with clinically localized disease, some men are still found to have locally advanced but non-metastatic disease as defined by high PSA (>20), high Gleason Scores (Gleason 8-10) and/or bulky palpable disease (9). Many of these men are treated with hormone

ablative therapy alone without the intent of cure. In the short term, this treatment will control disease as measured by a lowered or undetectable PSA and resolution or stabilization of local (urinary) symptoms if present. However, the emergence of hormone refractory disease occurs in nearly all patients, on average within 3 years. Since PSA is used to monitor the effectiveness of hormonal therapy, the detection of hormone refractory disease is now made considerably earlier than would be detected clinically by a positive bone scan or symptoms related to disease progression. Without further treatment these patients progress, with one third developing bone metastases and 20% dying from cancer within 2 years (10). Options for therapy are tertiary hormonal manipulations or taxane based chemotherapy which results in a 2-3 month enhancement of survival (11). This group of patients, much like patients with radiorecurrent disease need new options which can impact of both local and systemic disease.

IL-12 Gene Therapy

Interleukin-12 (IL-12) is a multi-functional cytokine that mediates a range of activities, which may be useful as an anti-cancer agent. Primarily, it directs Th1 differentiation of immune responses and enhances the proliferation and cytotoxicity of T-cells, natural killer (NK) cells, and macrophages, dependent to a large degree on production of IFN-γ by these effectors to maintain these activities (10-12). Tumor growth inhibition may also be attributed to the ability of IFN-γ to mediate anti-angiogenic activity through expression of inhibitors such as interferon-γ-inducible protein-10 (13-15). Several secondary mechanisms including downregulation of pro-angiogenic substances such as VEGF and matrix metalloproteinases (16), local cytotoxicity through production of nitric oxide (17) or influx of polymorphic neutrophils (PMNs) and macrophages (18-21) have all been implicated to mediate growth suppression. Together, these data indicate that IL-12 is a powerful oncologic compound.

However, use of recombinant IL-12 in patients has been associated with dose limiting toxicities of liver dysfunction and stomatitis without evidence of efficacy (32, 33). Gene therapy mediated-expression of IL-12 may be superior to intravenous recombinant product due to achievement of higher doses of IL-12 within an injected tumor without significant serum levels to result in toxicity. Furthermore, conceptually, high levels of IL-12 expression within the confines of a tumor and hence tumor antigen may provide the environment for a more efficient induction of immune responses. Indeed, IL-12 treatment through a variety of gene therapy approaches has demonstrated active growth inhibition of local tumors, suppression of both spontaneous and experimental metastases, and resistance to challenge tumor cell injections (15, 19, 20, 21, 24-32). As detailed below, in an aggressive orthotopic model of mouse prostate cancer injection of an adenovirus (Ad.) expressing mouse IL-12 (mIL-12) into a primary tumor resulted in local tumor growth suppression and enhancement of survival (33). Furthermore, Ad.mIL-12 treatment of the primary tumor also reduced the number of pre-established metastatic lung lesions, demonstrating significant

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